

CHEMOSYSTEMATICS AND ITS EFFECT UPON THE TRADITIONALIST¹

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What is a traditionalist, taxonomically speaking? I suppose a traditionalist might best be defined as a taxonomist trained as a pheneticist, practicing his trade as a pheneticist, and constructing his classification using primarily phenetic data. By this definition I am a traditionalist and consequently can claim to answer, *for myself*, the effect of chemosystematics upon my own traditional attitudes and outlooks. And this has been profound.

I say profound *not* because this new field has solved any large number of critical problems in plant taxonomy, but because where it has been used with skill and judgement, it has proved much more effective than phenetics in solving the particular problems concerned. Indeed, without chemical data many of the more intractable problems having to do with familial relationships among flowering plants generally are likely to remain unresolved: there are simply too many cooks and nearly all with varying tastes. Even if they all see the same phenetic substances in the phyletic cabinet, they nonetheless are prone to come up with different combinations of this or that ingredient (selected characters), with varying amounts (intuitive weighting), to say nothing of the condition (basic I.Q.) or temperature (zealousness) of the oven (i.e., brain).

I suspect that most traditionalists, even some of the best, do not like to be reminded that their approach is fraught with such variables, or that data derived from some other discipline might prove superior to those from their own.

As an example, when the late Dr. Alston and I first showed the utility of paper chromatography for resolving problems of natural hybridization in *Baptisia*, an eminent, not so classical, plant systematist suggested that our documentation of complex hybridization in this genus could have been accomplished with equal clarity using selected morphological characters arranged upon Anderson-type scatter diagrams. Needless to say this intellectual guffaw was issued by the late Edgar Anderson, and the ironic part of all this is that Anderson himself was the first to collect and call attention to the existence of hybrid swarms among this group of plants (Anderson, in Larisey, 1940b), but he failed to perceive its complexity, in spite of the fact that he collected his hybrid populations of *Baptisia* in a region where the potential for trihybridization is not infrequent (Alston & Turner, 1963). In fact, I seriously doubt that Anderson, or *any traditional* systematist, including myself, would have been able to recognize, much less intuit, trihybridization within this group, to say nothing of its documentation with reasonable certainty using morphological characters.

Trihybridization, of course, is rather the exception in nature: most species tend to comingle two at a time at any one site. But even then, lacking *in situ* clues (for example, two parental taxa occurring together with their putative

¹ Supported, in part, by NSF Grant DEB 76-09320.

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hybrids, as happens with *Baptisia* upon occasion), two-way hybrids may be difficult to detect, especially where these are quite distinct and relatively widely distributed. Hence we find the southeastern taxon, *Baptisia serенаe* Curtis, being recognized as a good species for over 125 years by a wide range of workers, including such an outstanding traditional worker as Wilbur (1963). It is, however, an F_1 hybrid of *B. tinctoria* (L.) Vent. \times *B. alba* (L.) Vent. A more remarkable F_1 hybrid, also long-recognized as a good species by nearly all traditionalists, including the only recent monographer of the genus (Larisey, 1940a) is *B. microphylla* Nutt., this being the relatively rare hybrid between *B. perfoliata* (L.) R. Br. and *B. lanceolata* (Walt.) Ell. While it is perhaps likely that these very distinct F_1 hybrids would have been detected if they were found growing with their putative parents, reasonable verification, short of long and laborious crossing experiments, would be difficult. If, however, their flavonoid profiles were sufficiently different, putative F_1 hybrids might be readily confirmed, even from hybrids mounted on herbarium sheets up to 100 years old.

Speaking of the superiority of micromolecular data for the resolution of systematic problems, the most telling example of its efficacy is that involving the detection of allopatric introgression. Edgar Anderson (1949) in an Epilogue to his brilliantly conceived text, *Introgressive Hybridization*, made the following statement:

How important is introgressive hybridization? I do not know. One point seems fairly certain: its importance is paradoxical. The more imperceptible introgression becomes, the greater is its biological significance. It may be of the greatest fundamental importance when by our present crude methods we can do no more than to demonstrate its existence. When, on the other hand, it leads to bizarre hybrid swarms, apparent even to the casual passer-by, it may be of little general significance Only by the exact comparisons of populations can we demonstrate the phenomenon The wider spread of a few genes (if it exists) might well be imperceptible even from a study of population averages, but it would be of tremendous biological import Hence our paradox. Introgression is of the greater biological significance, the less is the impact apparent to casual inspection.

In other words, in well-differentiated, sympatric species such as *Baptisia* where natural hybridization can be easily recognized and readily documented, its biological impact on evolutionary processes is negligible. But in allopatric situations where hybridization is very difficult to detect it is likely to be of the greatest biological significance.

In spite of these reflections from the foremost proponent of introgressive hybridization, few, if any, well-documented studies have been forthcoming on allopatric introgression. In fact, *the best documented* case in the literature for allopatric introgression is reportedly that involving *Juniperus virginiana* L. and *J. ashei* Buchh. (Anderson, 1953; Davis & Heywood, 1963). However, in a number of detailed studies, centered at The University of Texas (using 60 to 80 chemical characters as detected by gas chromatography as well as morphological characters which distinguish between the taxa), the existence of F_1 hybrids or their immediate derivatives could not be detected, even at sites where large populations of both species grew intermixed, and in *no instance* could the existence of introgression be inferred from the data accumulated (Flake, von Rudloff & Turner, 1969; Adams & Turner, 1970; Flake, von Rudloff & Turner, 1973). In short, what was taken to be a very well-documented case study of allopatric

introgression turned out to be a situation in which clinal intergradation in habitual features over a broad region occurred such that, *superficially*, hybridization and introgression might be inferred.

In hindsight, it now seems rather reasonable to have viewed the case study of introgression between *J. virginiana* and *J. ashei* with considerable doubt, for the two species are readily distinguished by a number of morphological features and are placed in different species—groups (sections) of the genus, and the character used for such taxonomic segregation (cilia along the leaf margins) does not, to our knowledge, segregate in putative hybrid swarms (i.e., the two species can always be recognized by this feature, and others, as attested to by the repeated correlation of this character with a plethora of chemical characters); other experienced field workers such as D. S. Correll (pers. comm.) have also had no difficulty in placing the plants concerned in one taxon or the other. In fact, as already indicated, the morphological variation found in *J. virginiana* is clinal, i.e., the species has formed or is in the process of forming regional races as a result of adaptational mechanisms arising out of its own gene pool, this being unrelated to the possible influx of genes from the largely allopatric *J. ashei*. Our work has substantiated fully these suppositions (Flake & Turner, 1973). Again, it is ironic that Hall, who was Anderson's student, should have documented introgressive hybridization where this was not occurring. We attribute this to the plasticity of the morphological characters selected for its detection. It was the absoluteness of the chemical data themselves which permitted resolution of the problem.

What we were left with then was no well-documented case study of allopatric introgression of a regional nature. Fortunately, however, there has been a recent, carefully conceived, populational study of *J. virginiana* and *J. scopulorum* Sarg. in the Missouri River Basin of the north central United States by Van Haverbeke (1968a) which appears to be a situation involving allopatric introgression of the type Anderson felt to be so important in evolutionary processes. The study seems to be unusually well documented. Van Haverbeke made very accurate records of the populational sites, including precise data on ten individually marked trees which were selected for study at each site. These included photographs and detailed field notes. In short, the *J. virginiana*-*J. scopulorum* complex appeared to provide an ideal case study of allopatric introgression using the chemonumerical methods that proved so effective in *disproving* the occurrence of this phenomenon in the *J. ashei*-*J. virginiana* "complex."

Van Haverbeke (1968a), through his study of these two taxa in the Missouri River Basin, has stated that:

The entire *Juniperus* population within the Basin is apparently of hybrid derivation with neither of the extreme parental types being found. There is a trend of increasing hybrid index values (also percentage germ plasm values) from southeast to northwest over the Basin from the reported range of *J. virginiana* to and into the reported range of *J. scopulorum*. This condition may be the result of bilateral introgression between the two species. There was, however, a strong tendency toward bimodality within the population as demonstrated by the presence of two distributions in each of the three hybrid indices. This indicated the presence of two different species—*J. scopulorum* and *J. virginiana*.

While Van Haverbeke (1968b) admits his data might be interpreted as

constituting evidence for introgression he, nevertheless, suggests, indeed champions, an alternate hypothesis:

As an alternative interpretation, it would seem that because of the greater diversity of the junipers in western North America, that *J. virginiana* was at some time derived from this area. It seems possible that with the inherent variability in the germ plasm ancestral to both *J. scopulorum* and *J. virginiana*, that propagules could flourish in sites toward the east. This could have initiated an eastward migration-propagule—which through mutation and selection eventually became what we now recognize as *J. virginiana*."

It should be noted that this latter evolutionary model is in direct conflict with that proposed by us (Flake, von Rudloff & Turner, 1969, 1973) in which we suggest that the Appalachian Region is the ancestral center for the origin of *J. virginiana* and its various races. Hence, the question of introgression between *J. virginiana* and *J. scopulorum* is left open by Van Haverbeke's study.

Initial investigation of the terpenes of *Juniperus scopulorum*, unlike that of *J. ashei*, showed that its volatile components were essentially those of *J. virginiana*, differing only in their quantitative expression. Subsequent populational analysis of the type employed in the *J. ashei*-*J. virginiana* studies showed that regional intergradation of the chemical characters occurred across the Missouri River Basin, much as found by Fassett (1944) and Van Haverbeke (1968a) for morphological features.

Three models might be proposed to account for the variation found in this region:

1. ANCESTRAL GENE POOL—*Juniperus scopulorum* and *J. virginiana* may have arisen from ancestral populations largely endemic to the Missouri River Basin. Subsequent evolutionary divergence to the west and east, respectively, might have occurred, leaving a residuum of genes common to each in the area concerned.

2. ALLOPATRIC INTROGRESSION—The variability is due to extensive gene flow from *J. scopulorum* into *J. virginiana* as a result of hybridization and backcrossing in peripheral regions of contact and areas of sympatry.

3. MIGRATORY TAILINGS—The River Basin was an ancestral migratory route through which *J. scopulorum*-like populations passed on their way to becoming what is now known in the eastern United States as *J. virginiana*. In Van Haverbeke's words (1968b), "Thus, rather than being considered as an introgressive series, this juniper population [those of the Missouri River Basin] can alternatively be interpreted as a divergent evolutionary series which has not yet completely separated."

It should be emphasized that in the investigation by Van Haverbeke about 40 morphological characters were selected for measurement and numerical analysis. These were obtained from some 700 trees from 72 sites scattered throughout the River Basin area. In spite of this excellently conceived, carefully documented, laborious study, the investigator was unable to decide, unequivocally, between models 2 and 3; in fact, he believed that his data best fit the migratory tailings model. (Model 1 was not tested, presumably because of its implausibility, considering the biogeographic history of the Basin region.)

Our own study (Flake, Urbatsch & Turner, 1978) also involved about 40 characters, all chemical. These were obtained from some 200 trees from 10 sites

systematically selected at about 150-mile intervals in a southeast-northwest transect across the Basin. In spite of the fewer populations sampled and the smaller overall sample size, we conclude our data overwhelmingly suggest that the variable River Basin populations are the result of allopatric introgression, primarily in the direction of *J. virginiana*, much as Van Haverbeke thought might be the case, but the morphological characters which he used were not sufficiently indicative to prove decisive.

MACROMOLECULAR APPROACHES

If I were interested in obtaining the most meaningful arrangement of present-day angiosperm families, *phylogenetically speaking*, I would rather have available to me the primary structure (amino acid sequence) of ten *metabolically important* enzymes (such as cytochrome *c*) of all of the taxa which comprise these groups than have a detailed listing of all of the exomorphic features which characterize the groups (Turner, 1969).

The nature and proper taxonomic position of the hypothetical past organisms that represent the branch points in the scheme cannot be determined solely from the phylogenetic relationships of modern species as deciphered from the amino acid sequences (Cronquist, 1976).

Protein sequencing and other molecular methods may, in fact, become in the near future the most powerful tools for the study of phlogeny (Ayala, 1976).

The amino acid sequence trees are obviously more compatible with some possible phylogenetic interpretations than others, or there would be no point in making them at all. If we assumed that they were in all respects correct insofar as they go, they would place certain limits on the general phylogenetic trees that could be seriously considered (Cronquist, 1976).

Though this be madness, yet there is method in't (Shakespeare, Hamlet, Scene II, Act 2).

. . . fossil evidence is highly in accord with an overwhelming mass of evidence from comparative morphology of living species that the Magnoliidae are the most primitive (i.e., least modified) group of living angiosperms . . . (Cronquist, 1976).

. . . the molecular tree indicates that present-day families represent relic groups which have for the most part had a long separate evolutionary history. They do not support the suggestion implicit in, for example, Cronquist's scheme . . . that the Magnoliidae gave rise to the Caryophyllidae on the one hand, and to the Rosidae on the other, the latter, in turn, giving rise to the Asteridae (Boulter, 1973).

Something is rotten in the state of Denmark (Shakespeare, Hamlet, Scene IV, Act 1).

With relatively few exceptions, the traditionalist might yawn at the seemingly trivial impact of micromolecular data upon his various systematic models. But he has not yet been able to treat with indifference the likely impact of macromolecular data upon his most treasured erection, the "Tree" to plant families. As unrecognizable as this tree might be to the various workers concerned, any reinsertion of branches or elevation of roots, using such chemical data, is met with alarming cries from this or that proponent. I refer specifically to the recent paper by Cronquist (1976) entitled, "The Taxonomic Significance of the Structure of Plant Proteins: A Classical Taxonomist's View." This is a 27-page rambling review covering the whole field of comparative enzymology, the gist of which is, because these data do not or have not supported my particular views, there must be something wrong with the approach.

The approach is the same as that which has been applied to animals successfully, namely, the use of amino acids among the homologous proteins in different

organisms as an indicator of time of branching. And, strangely enough, he accepts, in principle, the use of cytochrome *c* as a reasonable, but often unsteady, clock for animals, yet rejects this as valid for plants. I quote:

Given the difference in evolutionary pattern between plants and animals, it should not be surprising if the animal physiological system places stronger constraints on the acceptance of amino acid substitutions in cytochrome *c* than does the plant physiological system. It would be entirely in harmony with the other differences in plant and animal evolution if the same kinds of changes could be accepted by very different sorts of plants and if back mutations were not notably counter-selective.

Cronquist focuses his attack largely upon the data from Boulter's laboratory in Durham, England, which is the only group to sequence any significant number of plant proteins, namely plastocyanin and cytochrome *c*. Amino acid sequences from the latter, in particular, suggest that the familial tree is quite different from the one proposed by Cronquist (and, of course, that of Takhtajan, the two being quite similar). This is disturbing: everyone should accept that the Magnoliidae among the angiosperms is primitive to everything else. He does not like the Caryophyllidae coming off as a first branch on the cytochrome *c* familial tree. He does not like to think of the morphologically highly advanced Compositae represented as a very old isolated branch; everyone should know that it is recent, going back to the Miocene-Oligocene boundary (in spite of the fact that he acknowledges in footnote form that very recently published and unpublished pollen fossil data might push the family back to the Paleocene, if not earlier).

To me, it is remarkable that a traditionalist of his stature, fully aware of the ubiquity of this macromolecule among organisms generally and cognizant of its crucial role in the metabolic pathway of both plants and animals, should attribute the discrepancies to poor or erratic functioning of this kind of clock, rather than to the morphological data, which, after all, has no face, no dial, no nothing to suggest *the time* of branching of this or that phyletic line.

Cronquist (1976: 5), while accepting the general premise that the cytochrome *c* clock works for animals, nevertheless makes great gloat over the fact that the amino acid sequence of rattlesnake cytochrome *c* is out-of-line with the position of that organism in the phyletic tree. There follows a typical Cronquistian quote, "If the reported sequence for rattlesnake is correct, there seems to be no easy way to explain it, short of conjuring up a vision of a lonesome cowboy on the lone prairie, with none but a rattlesnake for company [referring to the seeming similarity of its sequence to that of the genus *Homo*]." I think that there are better ways to explain that single discrepancy, even *if* the sequence is correct.

Cronquist presumably wants us to believe that an occasional unsteady tick (if even that!) in the animal world is sufficient reason to believe that this same clock is *largely* unsteady in the plant world. In his desire to discredit such data, at least that of cytochrome *c*, he likens this to the Age and Area concepts of Willis (ludicrous!), followed by the statement that:

Evolution of other characters in both plants and animals tends to undergo periods of rapid radiation, interspersed with periods of more gradual change, and there is no *a priori* reason to suppose that adaptively significant changes in amino acid sequence would proceed any differently.

Of course, that's the point; there has been a sufficiently long record of plant evolution so as to believe that the cytochrome *c* clock has some kind of accuracy; fast or slow upon occasion, it nonetheless seems to average out as stochastic over time. Anyway, a generally erratic clock is better than no clock, giving the muddle of morphological darkness within which most plant taxonomists work.

As a final denouement, in case he hasn't convinced the reader, Cronquist adds a neat punch paragraph:

This discussion of the evolutionary clock may be something like beating a dead horse, but some people are still trying to ride the horse. If the horse is really dead it won't mind the beating.

One should perhaps remind Cronquist that, to judge from the recent articles by Fitch (1976), Zuckerkandl (1976), King (this symposium), and articles in press by yet other such workers, the horse is alive, is being ridden quite nicely, and perhaps doesn't deserve the beating being administered!

It would be unfair to conclude this address with the audience feeling that Cronquist might be quite negative towards the application of chemical data to taxonomic problems. He is not, for he concludes, in hindsight, that:

I welcome the appearance of amino acid sequences as an additional tool for taxonomists When we have the sequences for several proteins from members of a wide range of families, including critically important ones, we can make good use of this powerful tool.

Let's hope he means this; in the meantime he might wish to paraphrase Shakespeare's King Richard the Third, "A dead horse, a dead horse, *My Kingdom* for a dead horse!"

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